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Biomimetic Synthesis of Esters of Natural Amino Acids

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ABSTRACT: *A method for the synthesis of Gly, Ala, Phe, and Thr esters is proposed and considered as being a stage of possible biomimetic synthesis of peptides. The methyl esters of the said amino acids are obtained via intervention of 2-hydroxypropyl phosphonate. The resulting aminoacyl phosphonates reacts with methanol to produce the amino acid methyl esters, with the release of phosphoric acid. The reaction is carried out at room temperature in water.* © 2008 Wiley Periodicals, Inc. Heteroatom Chem 19:252–255, 2008; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20427

INTRODUCTION

The first biopolymers have been formed by condensation and dehydration in the primary ocean [1]. By the 60 years of the past century, the possibility of the polymerization of free and substituted amino acid phosphoanhydrides has been studied. The latter are energy-rich monomers, permitting the formation of peptide bonds even at high dilution and physiological pH and at room temperature [2–5]. The synthesis of peptides and proteins is one of the most important biosynthetic processes in living systems. Most of the amino acids are chemically activated via the formation of the respective aminoacyl adenylate, which contains a macroenergetic acyl phosphate bond. The carboxyl group is transferred to the hydroxyl one at the 3′-carbon atom in the ribose cycle of tRNA, resulting in the formation of an acyl ester and the release of the phosphate residue. In the end, peptization with a second amino acid at the active 2-hydroxy ester and release of the alcohol (ribose in the tRNA) occurs. In this paper, we propose a method for reproducing the biosynthesis of peptides. In the biochemical reactions with the participation of phosphates, the reaction center is the phosphate group. We have selected the starting phosphorus-containing compound 2-hydroxypropyl phosphonate, in which the substituents at the phosphoryl moiety and especially the presence of a vicinal hydroxyl group are the same as in 3′-position of ribose. It has long been known that 2-hydroxy esters of oxophosphorus acids can be used as phosphorylating reagents [6,7]. They are easy to obtain, simple compounds and have been successfully used in the phosphorylation of D-glucose [8], desoxynucleosides [9], and glycine [10].

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MATERIALS AND METHODS

The following compounds and materials, commercially supplied by Merck, Darmstadt, Germany were used: Gly, Ala, Phe, and Thr; phosphoric acid, in crystals; 1,2-propylene oxide; TLC aluminum sheets, and silica gel 60 F_{254}. NMR spectrometer Brucker Advance operating at 250 MHz was used.

RESULTS AND DISCUSSION

In our previous paper [10], we have reported that the reaction between 2-hydroxypropyl esters of oxophosphorus acids and glycine in methanol solution produces phosphoamidoacyl phosphates as shown in Fig. 1.

It could be expected that the available acyl–phosphorus bonds will be attacked by the alcohol to form acyl esters. To overcome the low solubility of the amino acids in methanol and to provide relevant conditions for comparison with the biochemical reaction, the esterification has been carried out in water in the presence of small amounts of methanol, just sufficient for the solubilization of the amino acids. As the starting phosphorus-containing compound, we have selected phosphorous acid, which ensures the possibility of tracking the changes in the reaction products by the changes in P—H in ^{1}H-NMR. On the other hand, the use of phosphorous acid avoids complications that would arise with the use of Mg-containing phosphoric acid derivatives, which are the corresponding reactants in live cells. The reaction between 2-hydroxypropyl phosphonate and the amino acid in water solution does not produce a phosphamide bond (P—N), but rather a salt-forming bond, (P—O^-—$^+NH_3$). The product, isolated by the reaction of alanine with 2-hydroxypropyl phosphonate, represents, after precipitation in ethanol, crystals with m.t. 208–210°C and NMR (DMSO): 7.901–5.417 d, $J_{P,H} = 621$ Hz (1H, PH); 3.77 m, $J = 7.2$ Hz (1H, CH); 1.47 d, 7.2 Hz (3H, CH_3).

$J_{P,H} = 621$ Hz is characteristic of salt-forming bonds. The compound is phosphoaminoalanil acylphosphonate (Fig. 2).

Owing to the presence of a salt bond between the acidic P-OH group and the amino group, the formed acylphosphate bond is stable even in the water solution. The presence of acylphosphate bonding offers the possibility of reaction with alcohols. The formation of the methyl esters of the amino acids is illustrated in Scheme 1.

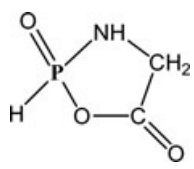


FIGURE 1 Phosphoamidoacyl phosphates.

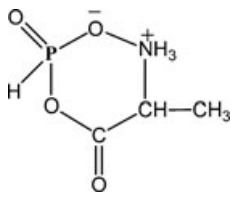


FIGURE 2 Phosphoaminoalanil acylphosphonate.

The first stage is the breaking of the zwitterion of the amino acid with the formation of a salt bond with the phosphonate. This is the reason for the better solubility of the products in the water/methanol solution.

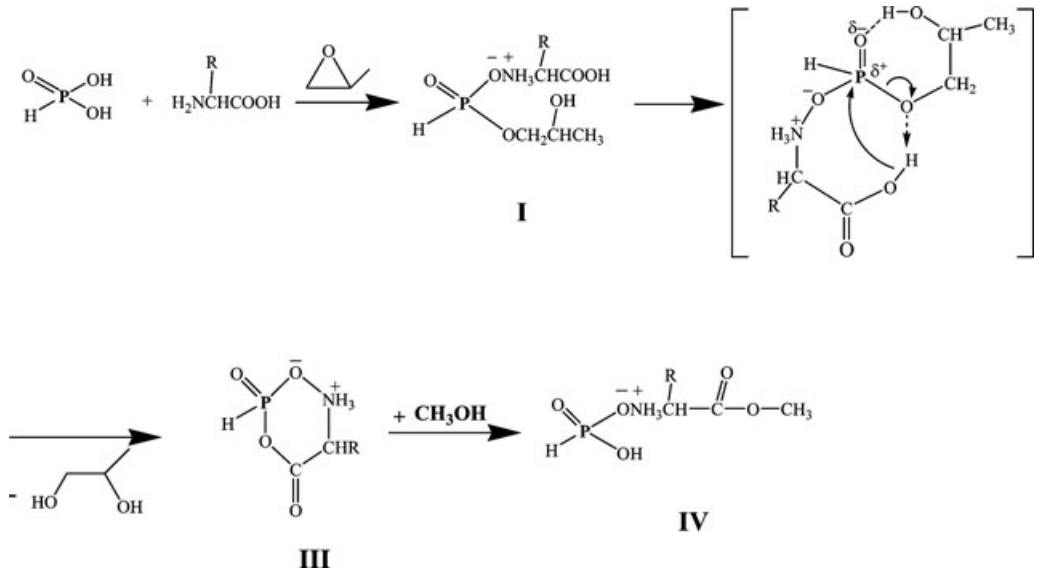
The second stage of the reaction involves a transition state, characterized by the possibility of the nucleophilic reaction at the phosphorus atom (compound II).

The third stage consists in the release of glycol and the formation of an acylphosphate bond, which is relatively stable in water because of the six atomic circles and the aminophosphoryl salt bonds.

In stage IV, methanol, which has nucleophile higher than water, attacks the acylphosphate bond with the formation of the methyl ester of the corresponding amino acid.

In practice, the amino acid and phosphorous acid were mixed in equimolar quantities, then dissolved in a methanol/water mixture and treated by the addition of 2–4 mol of propylene oxide. (According to weight analysis, 2 mol equivalents of propylene oxide reacted in the first stage.) The reaction was carried out for 1 h at room temperature and 15 min at 40°C and was monitored by the use of TLC. The final product was isolated from the reaction mixture in the following order:

1. acidification with 0.5 N HCl for breaking of the phosphamide bond. After evaporation, the residue is a white oil;
2. extraction with methylene chloride (chloroform, ethylacetate) for the removal of the remaining phosphorous acid and glycols;
3. removal of solvents by vacuum distillation;
4. addition of triethylamine, dissolved in methylene chloride, to liberate the amino acid ester from the hydrochloride;
5. extraction of the organic phase with water;
6. isolation of the final product.

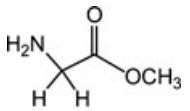
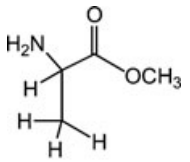
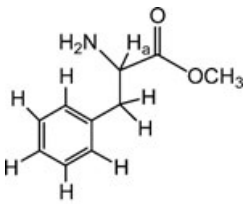
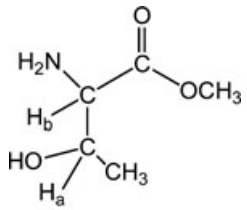


SCHEME 1 The formation of the methyl esters of the amino acids.

The esters were purified and isolated by column chromatography, using chloroform/methanol = 9:1. The products were characterized by ^{1}H- and ^{13}C-NMR (Table 1).

Amino acids are commonly esterified with alcohols in the presence of strong acids, ensuring acid catalysis; for example, alanine was mixed with methanol, saturated with hydrogen chloride [11]. A

TABLE 1 The Methyl Esters of Amino Acids and Its Characterization by ^{1}H- and ^{13}C-NMR

 **Gly.OMe**	**^{1}H-NMR ($CDCl_3$)**: 3.70 s, 3H, (OCH_3); 3.56 s, 2H, (CH_2) **^{13}C-NMR ($CDCl_3$)**: 175.2, (*C*=O); 44.3 (*C*H$_2$); 52.0, (O*C*H$_3$)
 **Ala.OMe**	**^{1}H-NMR ($CDCl_3$)**: 3.78 m, J = 7.2 Hz, 1H, (C*H*); 3,70 s, 3H, (OCH_3); 1.47 d, J = 7.2 Hz, 3H, (CH_3) **^{13}C-NMR ($CDCl_3$)**: 175.7, (*C*=O); 19.0 (*C*H$_3$); 50.3 (*C*H); 51.8, (O*C*H$_3$)
 **Phe.OMe**	**^{1}H-NMR ($CDCl_3$)**: 7.2 m, 5H, (C_6H_5); 3.75 dd, J = 5.2 Hz, 1H, (CH_a); 3.72s, 3H, (OCH_3); 3.07–3.14 dd, J = 5.2 Hz, 2.83–2.91 dd, J = 8.8 Hz, 2H, (CH_2); 1.72 s, 2H, (NH_2) **^{13}C-NMR ($CDCl_3$)**: 175, (*C*=O); 128, (*C*$_6$H$_5$); 55.5, (*C*H$_a$); 51.6, (O*C*H$_3$); 40.7, (*C*H$_2$)
 **Thr.OMe**	**^{1}H-NMR ($CDCl_3$)**: 3.86 m, J = 6.2 Hz, 1H, (H_b); 3.70 s, 3H, (OCH_3); 3.23–3.25 d, J = 5.1 Hz, 1H, (CH_a); 2.38 s, 3H, (NH_2, OH); 1.19–1.17 d, J = 6.3 Hz, 3H, (CH_3) **^{13}C-NMR ($CDCl_3$)**: 174.5, (*C*=O); 68.1, (*C*H$_b$); 59.8, (*C*H$_a$); 52.1, (O*C*H$_3$); 19.7, (*C*H$_3$)

more convenient preparative method is the one using thionyl chloride [12], where the mechanism of esterification is the same. We have carried out a blank experiment to establish whether the presence of phosphorous acid induced esterification without the introduction of propylene oxide in the system. Thus, equimolar quantities of phosphorous acid and phenylalanine were dissolved in the methanol/water mixture and was stirred for 1 h at room temperature and 15 min at 40°C. The mixture was titrated with 0.1 N NaOH to pH 5.88 (isoelectrical point of Phe) and precipitated in ethanol. The nonreacted phenylalanine was quantitatively isolated. No methyl ester was detected by TLC in the system.

It could be expected that the esterification in methanol solution (without water) would produce methyl esters of amino acids with higher yields. The control experiment with phenylalanine in methanol indicated that the yield of methyl ester of phenylalanine (45%) was less than in methanol/water. The reason of this result has not been studied in detail.

After the successful esterification of alanine, phenylalanine, and glycine, it was of interest to try the preparation of the methyl ester of an amino acid that has a hydroxyl group in the side chain. A possible attack of the side-chain OH group of the amino acid onto the acylphosphate bond could produce oligoesters of the amino acid. We have chosen threonine as the amino acid model for this purpose.

EXPERIMENTAL

Synthesis of the Methyl Ester of L-Phenylalanine

A flask equipped with a magnetic stirrer and a reflux condenser was loaded with 330 mg (2 mmol) L-phenylalanine and 164 mg (2 mmol) phosphorous acid. The solvent mixture of 2 mL water and 10 mL methanol was added and, after dissolution, 0.46 mL (6 mmol) propylene oxide was introduced. The system was stirred for 1 h at room temperature, then a second portion (2 mmol) of propylene oxide was added and the reaction mixture was stirred for 15 min at 40°C. Methanol was evaporated, and 1 mL 0.5 N HCl was added. The water was vacuum-evaporated, and the dry residue was washed with ether. The residue was then mixed with 5 mL methylene chloride and treated with 0.417 mL triethylamine, dissolved in 5 mL methylene chloride. The obtained solution was washed three times with 5 mL water. The organic phase was dried with $MgSO_4$. Methylene chloride was evaporated, and the dry residue was dissolved in a mixture of chloroform/methanol = 9:1 and was isolated by column chromatography. From the solution, 0.222 g methyl ester of phenylalanine crystallized (62% of the theoretical yield).

The methyl esters of glycine, alanine, and threonine were obtained by the same procedure. The esterification of threonine was performed with 3 mL water and 7 mL methanol.

Yields were Gly.OMe (67%), Ala.OMe (73%), and Thr.OMe (68%).

CONCLUSION

This method reproduces the biosynthesis of activated esters of amino acids. The authors consider it as the first stage of a biomimetic synthesis of peptides.

ACKNOWLEDGMENTS

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